

JAP20 Rec'd PCT/PTO 16 MAY 2006

**Immunoglobulin Preparations having increased stability****Description**

The present invention relates to a protein preparation having increased stability, comprising a stabiliser selected from the group consisting of non-polar and basic amino acids and having a pH of 4.2 to 5.4. The invention further relates to a pharmaceutical composition and a method of stabilising protein preparations.

Protein preparations, in particular immunoglobulin preparations for intravenous injection, have been in use for quite some time. Proteins, and immunoglobulin in particular, tend to form aggregates and/or dimers and to fragment or denature. If such solutions are injected intravenously, aggregates can give rise to severe side reactions including anaphylactic shock. In order to avoid aggregation, fragmentation, etc in such protein solutions and to improve their stability, a number of treatments have been tried in the state of the art. For instance, intravenous IgG for clinical use are often lyophilised (freeze-dried) for improved stability on storage, but such preparations must be reconstituted with a diluent before use. The reconstitution step is inconvenient and time consuming and increases the likelihood of contamination of the product. Another way of improving immunoglobulin stability and storage that is well known in the art is the addition of protein-stabilising excipients to the IgG preparation. Known excipients include sugars, polyols, amino acids, amines, salts, polymers and

surfactants. Such stabilisation strategies in protein pharmaceuticals are abundant in the art. For example, US Patent 4,499,073 (Tenold) improves the stabilisation through the selection of pH and ionic strength. JP 54020124 discloses the addition of an amino acid to an intramuscular preparation to render it storage stable and safe. JP 57031623 and JP 57128635 disclose the use of arginine and/or lysine with NaCl in 5 to 15% IgG preparations to achieve long-term stability in an intramuscular preparation. JP 56127321 discloses the addition of a sugar alcohol to IgG which works better than the previously used glucose in suppressing aggregation. JP 4346934 discloses the use of low conductivity (less than 1 mmho), pH 5.3 to 5.7 and optionally one or more stabilisers including PEG, human serum albumin and mannitol. US 4,439,421 (Hooper) teaches the addition of a hydrophilic macromolecule, a polyol and another protein to stabilise against ACA (anti-complement activity) generation. US 5,945,098 (Sarno) discloses the stabilisation of isotonic solutions by the addition of amino acids (0.1 to 0.3 M glycine), and non-ionic detergents (polysorbate) and PEG. US 4,186,192 (Lundblad) discloses various additives including amino acids, however, without specifying the use of single specific amino acids. This disclosure includes the stabilisation of IgG with maltose and additionally glycine to 0.1 M. US 4,362,661 (Ono) discloses the use of neutral and basic amino acids to impart stability on a 5% IgG product. All the above mentioned documents disclose IgG preparations of an acidic but still relatively high pH of above 5.2.

In addition to preventing the formation of immunoglobulin aggregates, it has also been recognised that dimer formation, in particular of IgG, can be detrimental to IgG preparations for intravenous use. Although IgG dimers are not known to cause anaphylactic shock, it has nevertheless been found that IgG preparations with a high dimer content are less well tolerated on intravenous injection and can give rise to undesirable side effects including fever, nausea and sometimes lowered blood pressure. Hypotensive side effects have been detected in a rat model by Bleaker et al. (Vox Sanguinis

52, 281-290, 1987), and this also shows an apparent correlation with the dimer content. Dimer formation is less of a problem when an IgG preparation is lyophilised shortly after it is produced. However, if the preparation is intended for storage in non-lyophilised liquid form, dimer concentration 5 increases with storage time.

10 US patent 5,871,736 (Bruegger et al.) discloses immunoglobulin preparations, particularly liquid preparations of IgG for intravenous infusion which comprise one or more amphiphilic stabilisers in order to stabilise against dimer formation. The amphiphilic stabilisers include nicotinic acid and its derivatives, in particular nicotinamide, and, mainly in conjunction with the above, amino acids having uncharged lipophilic side chains, e.g. phenylalanine, methionine, leucine, isoleucine, proline and valine. The experimental disclosure of this prior art document discloses amino acids 15 always in conjunction with nicotinamide, and the concentrations disclosed for the amino acids are 200 mmol/litre for proline, 80 mmol/litre for glycine and 120mmol/litre for isoleucine.

20 The pH range for the preparations disclosed in US 5,871,736 is broadly given as being between 4 and 8, but the actual disclosure of the Examples teaches a pH of 5.3.

25 Although the above US patent discloses IgG preparations in which dimer formation has been suppressed to a certain degree, it is still desirable to provide protein preparations, in particular immunoglobulin preparations, which show improved stabilisation, in particular at ambient temperature.

30 The inventors have found that a surprisingly high degree of stabilisation of liquid protein preparations can be achieved by adjusting the pH of the final preparation to between 4.2 and 5.4 and by adding as a stabiliser, a basic or non-polar amino acid.

Thus, the present invention provides a protein preparation having improved stability wherein the preparation comprises one or more stabilisers selected from the group consisting of non-polar and basic amino acids. Exemplary non-polar and basic amino acids, useful for the purposes of the present invention are histidine, arginine, lysine, ornithine (basic amino acids) and, isoleucine, valine, methionine, glycine and proline (non-polar amino acids).  
5 Particularly useful is proline. The stabiliser may be an amino acid of the group of non-polar or basic amino acids on its own, or it may be a combination of 2 or more such amino acids. The amino acids are preferably not used in combination with nicotinamide. The amino acid stabilisers may be natural amino acids, amino acid analogues, modified amino acids or amino acid equivalents. L-amino acids are preferred. When proline is used as the stabiliser, it is preferably L-proline. It is also possible to use proline equivalents, e.g. proline analogues.  
10  
15 Surprisingly, it was found that the addition of amino acids on their own, without other stabilisers (such as nicotinamide), and the adjustment of the pH of the final preparation markedly increases the stability of those preparations, particularly at ambient temperature. The increased stability is demonstrated by better stability of the preparations at temperatures between about 2°C and about 40°C, particularly at ambient temperature which preferably ranges from about 10°C, more preferably from about 15°C, more preferably from about 20°C to about 30°C, most preferably to about 25°C.  
20 The increased stability of the preparations of the invention is also visible at higher temperatures of about 30°C to about 40°C, including body temperature of about 37°C. Preferably, the increased stability is alternatively or additionally further defined as improved storage time, decreased fragmentation, decreased aggregate formation, decreased dimer formation or/and decreased discolouring. The improved storage time means that the  
25 preparations of the invention are preferably stable for at least 30 days, preferably at least 60 days, more preferably at least 90 days, more preferably at least 120 days, more preferably even longer than that.  
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Decreased aggregation preferably means that the preparations show a lower percentage of aggregates (in particular in case of Ig) than conventional preparations. Preferably, the dimer content of the preparations is below about 12%, preferably below about 10%, more preferably below about 8%.

5 Decreased colouring preferably means that the optical density of the formulations of the invention is between about 20 % and 60% lower than of conventional formulations.

In general, the protein preparations of the present invention are liquid formulations which are useful for intravenous injection. Such preparations can be stored and are stable in liquid form and thus do not require lyophilisation or other treatment and can be readily used.

10 Preferably, the protein preparation is an immunoglobulin preparation, in particular an antibody preparation wherein the antibodies may be of any idiotype but preferably IgG, IgA or IgM. IgG preparations are particularly preferred. The immunoglobulins can be polyclonal or monoclonal and can be isolated from human or animal blood or produced by other means, for instance by recombinant-DNA technology or hybridoma technology. In 15 general, immunoglobulins are obtained from blood plasma by alcohol fractionation, which may be combined with other purification techniques like chromatography, adsorption or precipitation. The immunoglobulins may be treated with trace amounts of enzymes (e.g. pepsin) in order to reduce anti-complementary activity or they may be used whole.

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25 The preparations can be obtained by methods known in the art, except that the pH of the final preparation is adjusted to a relatively high but acidic pH, namely in the range of about pH 4.2 to 5.4. It has been found that this pH range is particularly useful for improving the storage of characteristics of immunoglobulin preparations. The pH range is preferably from 4.5 to about 30 5.2, a pH range of about 4.6 to 5.0 being particularly preferred, pH 4.8 being especially preferred.

In the course of developing the preparations according to the present invention, it was also found that increasing the final concentration of the stabiliser allows a surprising improvement in the storage characteristics and 5 stability of the preparations. The stabiliser is therefore added to a final concentration of at least 0.2 M. Preferably, the final concentration is between 0.2 M and 0.4 M, more preferably between 0.2 M and 0.3 M, most preferably 0.25 M.

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The present invention is particularly useful for protein preparations with a relatively high protein concentration. The final preparation of the present invention has a protein concentration of about 5 to 25% w/v, preferably about 15 6 to 15% w/v, more preferably about 8 to 12% w/v, most preferably about 10% w/v. The final protein concentration will depend on various factors, such as the administration route, the type of condition to be treated, etc. The skilled person will be able to determine the optimal protein concentration for the intended application. For example, for intravenous infusion, the final preparation of the invention preferably has a protein concentration of about 20 15 to 20% w/v, preferably about 8 to 12% w/v. In the case of IgG for intravenous use, 10% w/v, i.e. 100g IgG/litre is particularly useful. For subcutaneous administration a higher dosage may be chosen, for instance about 15 to 20% w/v.

25 The present invention also provides a pharmaceutical composition comprising the protein preparation of the present invention as well as pharmaceutically acceptable additives. Such additives can be excipients, diluents such as water, and other substances such as non-buffering substances, for example sodium chloride, glycine, sucrose, maltose and 30 sorbitol. Such pharmaceutical compositions may be administered via various routes. For intravenous administration, a dosage of about 0.2g, preferably 0.5g to about 2.0g of immunoglobulin/kilogram of body weight per day may

be used.

A further aspect of the present invention is a method of stabilising protein preparations, in particular immunoglobulin preparations, comprising providing 5 an aqueous protein solution and adding one or more stabilisers selected from the group consisting of basic and non-polar amino acids, wherein the pH of the solution is adjusted to a pH of about 4.2 to 5.4. The pH is preferably adjusted to a value within the preferred ranges given above, pH 4.8 being particularly preferred. The method preferably comprises adjusting 10 the protein concentrations and stabiliser concentrations and choosing the stabiliser or stabilisers as stated above, proline being particularly preferred.

In particular, the method comprises the steps of providing an aqueous protein solution with a protein concentration of about 5 to 25 % w/v, adjusting 15 the pH of the solution to 4.2 to 5.4, and adding one or more stabilisers selected from the group listed above to the solution to give a final stabiliser concentration of 0.2 to 0.4 M to obtain a stable protein preparation. A number of processes are known to isolate immunoglobulins from human plasma or fractions thereof. Immunoglobulins can for example be purified by 20 cold ethanol fractionation and/or octanoic acid fractionation and/or chromatographic procedures. Purification methods that are particularly preferred for the purposes of the present invention include ethanol fractionation, followed by octanoic acid fractionation, followed by low pH treatment, chromatography and nanofiltration. In producing immunoglobulins 25 for intravenous applications such as those for the present invention, special care should preferably be taken to reduce or eliminate immune complexes with anti-complement activity and proteases like kallikrein or plasminogen. The protein to be used in the protein preparations of the present invention is brought to the desired concentration of between about 5 and 25% w/v by 30 known methods, e.g. by ultrafiltration. The pH of the liquid protein preparation is adjusted to a pH of 4.2 to 5.4, and the stabiliser is added to the solution at a final concentration of at least about 0.2 M. Preferably,

proline is used as the stabiliser, and it is preferably added at a concentration of about 0.2 M to 0.4 M, preferably about 0.25 M.

The present invention will now be illustrated by means of the following examples and figures.

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### **Brief Description of Figures**

Figure 1 shows the aggregate content as determined by HPLC for an 8% IgG solution containing either 0.25 M proline or 0.25 M glycine.

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Figure 2 shows the dimer content as determined by HPLC for an 8% IgG solution containing either 0.25 M proline or 0.25 M glycine.

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Figure 3 shows fragment content as determined by SDS PAGE of 8% IgG solutions containing either 0.25 M proline or 0.25 M glycine.

Figure 4 shows the optical density (UV350-500 nm) of two IgG solutions containing 0.25 M proline or 0.25 M glycine.

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### **Example 1 Manufacture of a protein preparation according to the invention.**

The starting material for the intravenous Ig manufacturing process is a licensed intermediate of the Kistler Nitschmann ethanol fractionation process. It is a precipitation of the immunoglobulin fraction from plasma using 19% ethanol at pH 5.8.

High molecular weight proteins, lipoprotein complexes, and other contaminants were precipitated using octanoic acid and were then separated via filtration in the presence of a filter aid. The supernatant was then concentrated before being subjected to a low pH incubation step.

The pH was then adjusted to pH 6.5 and the material further clarified by filtration to remove precipitated IgA and IgM. The IgG-enriched solution was then finally purified on an anion exchange resin, according to US 6,093,324, 5 except that the loading was 150g per litre resin.

Viral elimination was achieved by using a nanofilter.

Formulation: The nanofilterate was concentrated to 3% protein and diafiltered 10 against 5 volumes of water, followed by concentration of the IgG to 120 g per litre. Finally, the concentrate was stabilised with 250 mM L-proline, diluted to 100 g IgG per litre and the pH was maintained at pH 4.8. The formulated bulk was filtered through a 0.2  $\mu$ m membrane filter.

15 **Example 2 Testing of IgG preparations according to the invention.**

IgG concentrate, purified from plasma by a combination of precipitation steps and chromatography and virus inactivated according to Example 1 was split 20 into three portions with 260 ml formulated to pH 4.5, 420 ml formulated to pH 4.8 and 260 ml formulated to pH 5.1. The formulations were then divided, with one half being formulated with 0.25 M glycine and the other with 0.25 M proline. The final protein concentration was 8% w/v. Aliquots of 10ml were dispensed in 10 ml Type I glass vials (Type I rubber stoppers).

25 The aliquots were stored at three different temperatures, 2-8°C, 26°C and 45°C. The samples at 2-8°C were stored in the presence of light (Phillips TLD 18W/33). Samples were incubated at either 26°C or 45°C for at least two months in the dark. The results are shown in Figures 1 to 4.

30 **Aggregates**

The aggregate levels for IgG formulated with glycine were higher than those formulated with proline under all conditions tested.

Significant aggregate formation was promoted by incubation at 45°C. This 5 was similar for both proline and glycine formulations. Lower pH promoted aggregate formation at 45°C, with the pH 4.5 formulations containing 12.2% (proline) and 16.7% (glycine) aggregate at 90 days. In contrast, the pH 5.1 formulations contained 6.3% (proline) and 8.3% (glycine) aggregate at 90 days.

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#### Dimers

The dimer levels were influenced by pH, temperature and excipient type. The 15 pH proved the most important factor, with increased dimer levels observed as the pH of the formulation increased. This was observed for both glycine and proline formulations. The results indicate that formulations containing proline are capable of maintaining lower dimer levels than comparable glycine formulations. The incubation temperature modulates the 20 monomer/dimer equilibrium, with lower temperatures favouring the formation of dimers.

#### Monomers and Dimers

25 The combined monomer/dimer content for all formulations at 2-8°C and 26°C remained above 90%. Lower levels were observed in IgG solutions formulated with glycine due to their higher aggregate content. Incubation at 45°C resulted in three formulations having levels below 90% after 60 days (85.1% glycine, pH 4.5, 89.1% proline, pH 4.5 and 89.1% glycine, pH 4.8). 30 Again, these results highlight the increased ability of proline over glycine to preserve the molecular integrity of IgG molecules.

### IgG Fragments

5 The results indicate that the glycine formulations contain slightly lower fragment levels as compared to proline. Incubation temperature and pH proved to be the most important factors influencing IgG fragmentation. At 45°C the fragment levels for proline formulations range from 5.2% (pH 5.1) to 5.8% (pH 4.5); while the glycine formulations ranged from 4.3% (pH 5.1) to 4.8% (pH 4.8). At elevated pH (4.8 – 5.1) there was less fragmentation.

### 10 Appearance of the solution

Four main parameters were investigated: clarity, turbidity, particles and visible colouring. Parameters such as appearance, clarity and turbidity were satisfactory. Colouring (yellow/brown) of the solutions occurred during the 15 incubation period and was related to both the incubation temperature and light exposure. The colouring of the IgG formulations was monitored using the optical density test (UV350-500 nm). Increased colour was associated with exposure to light and increased incubation temperatures. The glycine formulations exhibited optical densities that were between 25% and 48% 20 higher than the corresponding proline formulations. These results provide further evidence that proline is a better stabiliser than glycine in IgG solutions. At elevated pH (4.8 – 5.1) there was less colouration than at lower pH (4.5).

### 25 Example 3 Stability of IgG preparations according to the invention (pH dependence).

IgG concentrate, purified from plasma by a combination of precipitation steps and chromatography and virus inactivated according to Example 1 was split into two portions and formulated with or without 400 mmol/L L-proline at pH 30 4.2, 4.8, 5.3 and 6.8. The final protein concentration was 12% w/v. Aliquots

of 10 ml were dispensed into glass vials and incubated at 40 °C for at least 3 months in the dark. At time 0 and after 90 days incubation samples were analysed by HPLC for aggregates; dimeric, monomeric IgG, by photometry for absorbance at 350 – 500 nm, by SDS PAGE (fragments) and specific antibodies directed against hepatitis virus B surface antigen (anti-HBs).

5 The results presented in Table 1 show that best stability of the IgG solution is obtained at a moderate acidic pH of 4.8 to 5.3.

Table 1: pH dependence of the stability of a protein preparation (10%) according to the invention

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Additive	none							
	4.2		4.8		5.3		6.8	
pH	0	90	0	90	0	90	0	90
Incubation time (days)	0	90	0	90	0	90	0	90
Aggregate (%)	3.5	40.2	1.16	5	1.31	3.1	3.22	2.7
Dimer (%)	6.5	3.6	10.6	11.1	12.2	13.8	16.4	19.0
Fragments (%)	1.4	2.6	1.3	3.5	1.3	3.6	1.5	3.4
Absorbance (350-500nm)	0.107	0.159	0.125	0.186	0.156	0.205	0.355	0.936
anti-HBs (IU/ml)	7.0	2.6	6.5	3.5	6.3	3.5	6.3	3.5

Additive	L-Proline (400 mMol/L)							
	4.2		4.8		5.3		6.8	
pH	0	90	0	90	0	90	0	90
Incubation time (days)	0	90	0	90	0	90	0	90
Aggregate (%)	1.97	26.4	0.82	4.5	0.85	2.5	1.78	2.9
Dimer (%)	4.3	4	6.4	6.2	7.8	9.5	11.8	13.9
Fragments (%)	1.4	2.9	1.3	3.5	1.3	4.0	1.5	3.6
Absorbance (350-500nm)	0.202	0.234	0.134	0.213	0.125	0.235	0.249	0.55
anti-HBs (IU/ml)	7.0	2.9	6.5	3.5	6.3	4.0	6.3	3.6

**Example 4 Stability of IgG preparations according to the invention formulated with different additives.**

IgG concentrates, purified from plasma by a combination of precipitation steps and chromatography and virus inactivated according to Example 1, 5 were formulated with additives of different substance classes (sugars and sugar alcohols, amino acids, detergents) at pH 4.2, 4.8, 5.3 and 6.8. The final protein concentration was 10% w/v. Aliquots of 10 ml were dispensed into glass vials and incubated at 37°C or 40 °C for at least 3 months in the 10 dark. After 90 days incubation samples were analysed by HPLC for aggregates, dimeric, monomeric IgG and fragments, by photometry for absorbance at 350 - 500 nm and by ELISA for specific antibodies directed against hepatitis virus B surface antigen (anti-HBs).

The results presented in Table 2 show that best stability of the IgG solution is 15 obtained at a moderate acidic pH of 4.8 or 5.3 with the most favorable formulations with L-proline.

**Table 2: Stability of a protein preparation (10%) according to the invention formulated with different additives and at different pH.**

**pH 4.2**

Inc. Temp.	Additive	Absorbance	HPLC				anti-HBs IU/mL
			350-500nm	Aggregates %	Dimers %	Monomers %	
37°C	D(-)Mannit (10%)	0.134	3.00	4.19	87.37	5.44	1.63
	Saccharose (10%)	0.271	3.18	3.38	87.87	5.57	1.59
	Maltose (10%)	0.422	5.30	3.83	85.54	5.33	1.42
	Glycine (250 mmol/L)	0.177	5.82	3.75	84.64	5.79	1.57
	L-Proline (250 mmol/L)	0.166	5.50	2.98	85.87	5.84	1.70
	Polysorbat 80 (0.02%)	0.166	7.03	3.54	83.94	5.49	1.53
	none	0.172	7.83	3.67	83.11	5.39	1.52
40°C	Glycine (400 mmol/L)	0.251	22.06	5.27	68.59	4.08	2.44
	L-Proline (400 mmol/L)	0.231	26.38	3.96	65.77	3.89	2.87
	L-Isoleucine (200 mmol/L)	0.257	52.03	2.59	41.25	4.13	1.74
	L-Methionine (200 mmol/L)	0.175	37.66	3.32	55.41	3.61	2.44
	L-Valine (250 mmol/L)	0.197	29.30	4.40	62.67	3.63	2.66

Table 2 continued

pH 4.8

Inc. Temp.	Additive	Absorbance	HPLC				anti-HBs
		350-500nm	Aggregates %	Dimers %	Monomers %	Fragments %	IU/mL
37°C	D(-)Mannit (10%)	0.147	3.45	7.08	86.84	2.62	1.93
	Saccharose (10%)	0.195	0.73	6.07	90.24	2.96	1.66
	Maltose (10%)	0.489	0.97	7.66	88.41	2.96	1.73
	Glycine (250 mmol/L)	0.242	1.37	7.05	88.64	2.94	1.94
	L-Proline (250 mmol/L)	0.183	0.99	4.90	91.17	2.94	2.25
	Polysorbat 80 (0.02%)	0.166	1.25	7.14	88.75	2.86	1.94
	none	0.165	1.29	7.54	88.30	2.87	1.94
40°C	Glycine (400 mmol/L)	0.241	3.79	10.42	83.47	2.32	3.77
	L-Proline (400 mmol/L)	0.213	4.47	7.09	85.96	2.48	3.54
	L-Isoleucine (200 mmol/L)	0.488	4.88	9.67	83.27	2.18	3.87
	L-Methionine (200 mmol/L)	0.174	5.93	7.46	84.08	2.53	3.83
	L-Valine (250 mmol/L)	0.207	6.48	9.58	81.53	2.41	3.71

pH 5.3

Inc. Temp.	Additive	Absorbance	HPLC				anti-HBs
		350-500nm	Aggregates %	Dimers %	Monomers %	Fragments %	IU/mL
37°C	D(-)Mannit (10%)	0.179	1.39	10.49	85.62	2.50	1.84
	Saccharose (10%)	0.185	0.61	8.46	88.36	2.57	1.88
	Maltose (10%)	0.516	0.76	11.04	85.51	2.69	1.70
	Glycine (250 mmol/L)	0.263	0.98	9.09	87.59	2.34	1.92
	L-Proline (250 mmol/L)	0.195	0.78	7.34	89.58	2.30	2.20
	Polysorbat 80 (0.02%)	0.196	0.94	9.56	86.94	2.56	1.91
	none	0.177	0.93	10.13	86.40	2.53	1.90
40°C	Glycine (400 mmol/L)	0.336	2.82	12.75	82.43	2.00	3.92
	L-Proline (400 mmol/L)	0.235	2.49	9.54	85.90	2.07	4.02
	L-Isoleucine (200 mmol/L)	0.275	4.14	11.06	82.76	2.04	3.73
	L-Methionine (200 mmol/L)	0.207	3.21	9.67	84.42	2.71	3.58
	L-Valine (250 mmol/L)	0.253	4.26	12.00	81.30	2.44	3.93

Table 2 continued

pH 6.8

Inc. Temp.	Additive	Absorbance 350-500nm	HPLC				anti-HBs IU/mL
			Aggregate %	Dimer %	Monomer %	Fragment %	
37°C	D(-)Mannit (10%)	0.300	4.94	12.14	80.25	2.67	1.61
	Saccharose (10%)	0.270	0.95	12.19	84.16	2.70	1.89
	Maltose (10%)	1.008	5.96	16.81	74.46	2.77	1.34
	Glycine (250 mmol/L)	0.807	1.19	12.34	84.39	2.08	1.87
	L-Proline (250 mmol/L)	0.328	1.10	10.89	85.87	2.14	1.90
	Polysorbat 80 (0.02%)	0.308	1.50	13.85	81.74	2.92	1.60
	none	0.344	1.40	13.68	81.91	3.00	1.73
40°C	Glycine (400 mmol/L)	1.063	3.00	16.61	78.08	2.32	3.72
	L-Proline (400 mmol/L)	0.550	2.89	13.95	80.69	2.47	3.61
	L-Isoleucine (200 mmol/L)	0.840	4.47	15.38	77.77	2.38	3.87
	L-Methionine (200 mmol/L)	0.687	2.96	13.68	79.79	3.57	3.66
	L-Valine (250 mmol/L)	1.083	4.62	15.16	75.33	4.89	3.13

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